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Antecedents of Severe and Nonsevere Medication Errors

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Abstract

Purpose—To investigate if differences in antecedents of severe and nonsevere medication errors exist.

Design—A longitudinal study of 6 months of data from 279 nursing units in 146 randomly selected hospitals in the United States (US).

Methods—Antecedents of severe and nonsevere medication errors included work environment factors (work dynamics and RN hours), team factors (communication with physicians and nurses' expertise), person factors (nurses' education and experience), patient factors (age, health status, and previous hospitalization), and medication-related support services. Generalized estimating equations with a negative binomial distribution were used with nursing units as the unit of analysis.

Findings—None of the antecedents allowed predicting both types of medication errors. Nurses' expertise had a negative and medication-related support services had a positive association with nonsevere medication errors. Nurses' educational level had a significant nonlinear relationship with severe medication errors only: As the percentage of unit BSN-prepared nurses increased, severe medication errors decreased until the percentage of BSN-prepared nurses reached 54%. In contrast, RN experience had a statistically significant relationship with nonsevere medication errors only and nursing units with more experienced nurses reported more nonsevere medication errors.

Conclusions—Severe and nonsevere medication errors might have different antecedents.

Clinical Relevance—Error prevention and management strategies should be targeted to specific types of medication errors for best results.

Keywords

Severe medication errors; nonsevere medication errors; nurses' education and experience

Despite increased attention to medication errors during the past decade, the incidence of medication errors has remained relatively unchanged. In 1995, one study reported that 2% to

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14% of patients in the US experienced at least one medication error during hospitalization, which is equivalent to 0.3 errors per patient-day (Bates, Boyle, Vander Vliet, Schneider, & Leape, 1995). Eleven years later, an Institute of Medicine (2006) report indicated that at least one medication error occurs every day for every hospitalized patient, suggesting that medication errors remain a significant problem. The situation is similar in other countries. Researchers from Europe, Asia, and Australia have also reported a high incidence of medication errors, including incorrect labeling, wrong rate of drug administration, and deviation from aseptic technique when administering intravenous medications (Cousins, Sabatier, Begue, Schmitt, & HoppeTichy, 2005; Haw, Dickens, & Stubbs, 2005; Ito & Yamazumi, 2003; Lin, Liao, Cheng, Wang, & Hsueh, 2008; Runciman, Roughead, Semple, & Adams, 2003). Consequently, continued research to better understand factors that contribute to the occurrence of medication errors remains a priority.

Previous work on predictors of medication errors has often been focused on identifying factors that are difficult to modify. These include hospital characteristics such as number of hospital beds, teaching status, or geographic region (Brennan et al., 1991; Thornlow & Stukenborg, 2006) and patient risk factors such as age, sex, or medical diagnosis (Bates, Miller, et al., 1999; Evans, Lloyd, Stoddard, Nebeker, & Samore, 2005). Other research has been focused on the relationship between nurse staffing and medication errors and indicated that better staffing is associated with fewer medication errors (Blegen, Goode, & Reed, 1998; McGillis Hall, Doran, & Pink, 2004).

One of the limitations of these studies is that many investigators have implicitly assumed that all medication errors are the same in terms of severity. In fact, the Joint Commission (JCAHO) indicates certain medication errors as sentinel events, defined as medication errors that lead to death or serious physical or psychological injury, and some researchers have focused on sentinel events. However, the primary interest of such studies is to find root causes of the event retrospectively, rather than examining nonsentinel events, which are far more common (e.g., Rex, Turnbull, Allen, Vande Voorde, & Luther, 2000). If errors of different levels of severity have different underlying mechanisms, they might require different methods of risk management (Reason, 1995). Further, if errors of different severity have distinctive predictors, research that combines them into a single category could yield misleading findings.

Therefore, the purposes of this study were to examine nursing-unit characteristics contributing to medication errors at acute-care hospitals and investigate whether medication errors of different levels of severity have different antecedents. In the current study, medication errors were categorized into two types: severe errors that required immediate clinical attention and interventions resulting from potential deterioration in patient conditions and nonsevere errors that did not require such attention or interventions.

Error-Producing Conditions

We developed a model of antecedents of medication errors based on the conceptualization of error- producing conditions that Dean, Schachter, Vincent, and Barber (2002) suggested: work environment factors, team factors, person factors, and patient-specific factors.

Medication-related support services were added because previous investigators have reported the importance of technologies such as computerized physician order entry or unit-dose systems (Bates, Teich, et al., 1999; Kaushal, Shojania, & Bates, 2003).

Work-environment factors were specified as work dynamics and RN hours. Highly dynamic work situations (i.e., frequent changes of orders, care plans, and procedures) create conditions in which nurses might be prone to making medication errors. In such situations, nurses might be frequently interrupted and become easily distracted, forget what they were doing, and be more likely to make errors (Cohen, Robinson, & Mandrack, 2003). Fewer RN hours of care have also been reported to be associated with increased medication errors (Blegen et al., 1998; McGillis Hall et al., 2004). On a nursing unit with more RN hours, there is an increased level of redundancy in the surveillance and supervision of each other's performance and thus fewer chances for errors.

Team factors included nurses' communication with physicians and nurses' expertise. Manojlovich and DeCicco (2007) recently found that as nurses' perceptions of their communication with physicians improved, their reported medication errors decreased. The quality of nurse- physician communication has also been related to the "quality of drug use," evaluated as proper drug selection and the absence of unnecessary polymedicine (i.e., concurrent use of three or more drugs; Schmidt & Svarstad, 2002).

In contrast, failures of communication, particularly those resulting from inadequate "hand offs" between care providers (Bates & Gawande, 2003), verbal miscommunication (Donchin et al., 1995), and impaired communication between nurses and physicians (Rosenstein & O'Daniel, 2005) have been found to increase medical errors. Another team factor examined is nurses' expertise, defined as the ability to recognize potentially ominous events early (Minick & Harvey, 2003). Although not required, expert nurses oversee other nurses' work as well as their own, and are always aware of what is going on in their nursing unit. They are vigilant in recognizing changes in a patient's condition and take action in a timely manner so they can prevent near misses from becoming accidents. Therefore, a nursing unit staffed with such nurses will likely have fewer medication errors.

The person factors examined in this study were nurses' education and experience. Nurses with limited experience or education are more likely to make errors because of insufficient skills and inadequate knowledge of pharmacology. They accumulate medication-related skills and knowledge through further education and experience as an RN (Ives, Hodge, Bullock, & Marriott, 1996).

However, the effect of higher education and more experience is not likely to continue to increase at a constant rate because when nurses gain confidence with their knowledge and skills, they might not be as attentive to procedures they have performed over and over. In fact, experienced and educated nurses are more likely to recognize a pattern and act according to a schema (i.e., an organized memory "template") applied to the pattern, rather than analyzing each new problem separately. These attributes are more likely to result in "strong-but- wrong" errors, in which experienced nurses tend to be more certain, although wrong, in their judgment about the appropriateness of the drug dose ordered when compared to inexperienced nurses (Ferner & Aronson, 2006; Perlstein, Callison, White, Barnes, & Edwards, 1979; Reason, 1990). Therefore, we argue that the relationship between nurses' experience and education and medication errors will be linear up to a point and then plateau.

A nursing unit in which various types of medication- related support services are available is less likely to have medication errors (Kaushal & Bates, 2002; Oren, Shaffer, & Guglielmo, 2003). Such support services include both high-tech (i.e., computerized physician order entry systems, unit-dose medication systems, automated medication administration systems) and low-tech services (i.e., transcribing orders and placing information in patient charts, intravenous (IV) team services, medication and IV fluid-delivery services, pharmacist consultation). Because these medication-related services are designed to standardize and simplify the medication procedures nurses use, they can decrease the possibility of human error at each point in the medication process.

Patient characteristics of age, health status, and previous hospitalization have been associated with increased medication errors. Although some researchers have found no relationship between patient age and medication errors (e.g., Bates, Miller et al., 1999; Evans et al., 2005; Hicks, Becker, Windle, & Krenzischek, 2007), in general, investigators have suggested that patient age is positively related to medication errors. While health status and previous hospitalization have not been directly linked to medication errors, they are considered as proxy measures of patient comorbidities, which have been found to be associated with increased risk of adverse drug events (Evans et al., 2005).

Methods

Data and Sample

The data for this study were derived from the Outcomes Research in Nursing Administration Project (ORNA-II), a multisite organizational study. The purpose of this parent study was to investigate relationships among hospitals' external and internal environments, staffing adequacy, work conditions, and organizational and patient outcomes (Mark et al., 2007). ORNA-II data were collected for 6 months from 146 hospitals randomly selected from the JCAHO-accredited acute care facilities with at least 99 licensed beds. Two medical-surgical units or medical-surgical specialty units (e.g., orthopedic, neurology, telemetry, step-down) at each hospital participated in the parent study (*N*=286). Federal, psychiatric, and for-profit hospitals were excluded, as were critical care, pediatric, labor and delivery, and psychiatric units. Registered nurses employed on their units for more than 3 months were eligible to participate (*N*=4,954).

Because power calculations and sample size determinations are not well formulated for GEE, especially with negative binomial regression models, we report power for the parent study. We calculated power using the approach of MacCallum, Browne, and Sugawara (1996), in which power is based on the ability to distinguish a good-fitting from a poor-fitting model using the root mean square error of approximation (RMSEA). The number of nursing units in the secondary analysis reported here— 286—meets this criterion.

Procedures

At each hospital, a study coordinator was appointed who was in charge of distributing questionnaires to staff nurses and obtaining administrative data. To enhance consistency in data-collection procedures and data integrity, the coordinators participated in 1½ days of training by the study project team. Purposes of the training workshop were to introduce the study and team members, present the study aims and goals, review and clarify conceptual and operational definitions for key data elements, describe detailed procedures for data collection, and share successful data collecting strategies.

Staff nurses on each nursing unit completed three questionnaires distributed during the 1st, 3rd, and 5th month of data collection. Data on education, experience, work dynamics, and expertise were obtained during the 1st month of data collection. Study coordinators selected 10 patients at random from each nursing unit and the patients provided data during the final month of data collection. The study coordinator provided data monthly for 6 months on unit-level RN hours, patient days, and both types of medication errors.

Measures

Dependent Variables

A medication error was conceptually defined as an error in administering, rather than in prescribing medication. Operationally, we defined medication errors as the wrong dose, wrong patient, wrong time, wrong drug, wrong route, or omission. We measured the number of medication errors over 6 months; this information was derived primarily from incident reports. Medication errors that resulted in increased nursing observation or technical monitoring, laboratory or radiographic testing. medical intervention, or transfer to another unit were classified as severe medication errors and the rest as nonsevere medication errors.

Independent Variables

Work dynamics—Work dynamics were measured by a seven-item Likert-type questionnaire in which nurses were asked about the extent to which their units were characterized by frequent interruptions or unanticipated events (Salyer, 1996). Items on this scale were anchored by six response options ranging from *strongly disagree* to *strongly agree*, with higher scores indicative of greater work dynamics (Cronbach's alpha=0.85). This scale had one factor explaining 53.8% of variance in work dynamics.

RN hours—RN hours were defined as the percentage of nursing-care hours delivered by RNs (i.e., permanent, float, per diem, and agency RNs) among those delivered by all nursing personnel.

Communication with physicians—Communication with physicians was measured by using the Relational Coordination Scale (Gittell et al., 2000). The original scale is a 5-point Likert-type scale in which healthcare providers in various disciplines are asked to assess the quality of their collaboration with each of eight other disciplines regarding four communication dimensions (frequency, timeliness, accuracy, and problem-solving) and three relationship dimensions (shared goals, shared knowledge, and mutual respect). Higher scores were indicative of better communication and better relationships (Cronbach's alpha=0.82). This scale consisted of two factors representing communication and relationship dimensions and together these factors explained 65.0% of variance in communication with physicians.

Nursing expertise—Nursing expertise was measured with eight items from the Nursing Expertise and Commitment to Care Scale (Minick, Dilorio, Mitchell, & Dudley, 2000). RNs were asked to rate the expertise of their nursing work- group in terms of recognizing critical patient problems. Higher scores were indicative of greater expertise (Cronbach's alpha=0.92). This scale had one factor explaining 65.2% of variance in nursing expertise.

Education level—Education level was defined as the proportion of nurses on each nursing unit whose highest education level was a bachelor's degree or higher.

Experience—Experience was defined as the average of each nurse's experience as an RN in months. To investigate a diminishing marginal effect of education and experience levels (i.e., allowing for an increase in education and experience to have a different effect on outcomes when the levels of education and experience are lower or when they are higher), both linear and squared terms were included.

Medication-related support services—Medication-related support services were measured by using the sum of scores on a checklist in which nurses rated six medication-related support services as not available, inconsistently available, or consistently available (Mark, 1992; Mark, Salyer, & Wan, 2003). Higher scores indicated greater availability of these services. Intraclass correlation (ICC) measures were used to examine interrater reliability and were 0.26 and 0.86, respectively.

Patients' age—Patients' age was defined as the average age of patients who completed the questionnaire on each unit.

Health status—Health status was patients' perception of their health status. Patients were asked to rate health status in five categories from *very poor* to *very good*.

Previous hospitalization—Previous hospitalization was a dichotomous variable to determine if the patient had been hospitalized in the past year. "Yes" was coded as 1.

Because the unit of analysis was nursing units, some variables (e.g., work dynamics, communication with physicians, nursing expertise) measured at the individual level needed to be aggregated to represent measurement at the nursing-unit level. Theoretical justification for data aggregation is that these variables were measured with self-administered questionnaires in which staff nurses responded to items referenced to their nursing unit. Methodological justification for data aggregation was based on the r_{wg} statistic, which is an estimate of within-group agreement (James, Demaree, & Wolf, 1984). All variables showed r_{wg} greater than 0.70, indicating adequate within-unit agreement (Klein & Kozlowski, 2000).

Data Analysis

To model the two types of medication errors during 6 months, generalized estimating equations (GEE) with a negative binomial distribution were used. This method accounted for three issues present in the data: the nature of the count variable (i.e., medication errors), possible correlations among repeated observations, and overdispersion problems, which are frequent in count data (Liang & Zeger, 1986). To fit a log-linear model to the ratio of medication error incidents to patient days (Stokes, Davis, & Koch, 2001), the natural logarithm of patient days was used as an "offset" term. Because nursing-unit data were nested within hospital data, hospital random effects were applied to account for nursing-unit correlation.

Results

Table 1 shows a summary of descriptive statistics for the sample. A total of 1,671 observations were used in the model. On average, nursing units in this study had 0.61 severe (range=0 to 15 errors) and 3.86 nonsevere (range=0 to 38 errors) medication errors per month. Nursing units had 27 on the work dynamics scale (range of 16 to 37) and 62% of RN hours (range of 27% to 100%), meaning that 62% of nursing-care hours were provided by RNs. On average, 37% of the RN staff reported being prepared at the baccalaureate level (range=0% to 100%), and the nurses' average experience was 138 months (range=44 months to 323 months). Concerning patients' characteristics, the average age was 57 years old (range=37 to 78) with health status rated as moderate to good, and 53% (range 0% to 100%) of the patients had experienced hospitalizations in the past year. Overall correlations among the independent variables ranged from −0.41 to 0.27 (results not shown).

Table 2 shows the results of GEE analysis. None of the antecedents allowed for predicting both types of medication errors. The two work-environment factors (i.e., work dynamics and RN hours) were not significantly related to either type of medication errors.

Among the team factors, nursing expertise had a statistically significant negative association with nonsevere medication errors indicating that the greater the level of nursing expertise, the fewer nonsevere errors ($p<0.01$). However, this relationship did not hold true for severe medication errors; although not significant, the direction was positive. Communication with physicians was not statistically significant.

Concerning the person factors, nurses' educational level had a significant nonlinear relationship with severe medication errors only ($p<0.01$). This indicates that as the percentage of BSN-prepared nurses on the nursing unit increased, severe medication errors decreased. To examine the nonlinear relationship, a derivative of the linear and squared coefficients for the education equation was calculated. Using this value, which was 54%, we found that above this point, severe medication errors no longer decreased. In contrast, nurses' experience had a significant nonlinear relationship with nonsevere medication errors only and the shape of the relationship was opposite to that of nurses' educational level ($p<0.01$). In other words, as nursing units had more experienced nurses they reported more nonsevere medication errors. Medication-related support services were significantly positively associated with nonsevere errors ($p<0.01$), indicating that the more medication-related support services were available the more nonsevere medication errors nursing on units. However, this factor did not have a significant association with severe medication errors. No patient characteristic (i.e., age, health status, and previous hospitalization) allowed predicting either type of medication error.

Discussion

The current study indicated whether antecedents of severe and nonsevere medication errors differed from one another. We found that none of the antecedents predicted both types of medication errors and some had a positive association with one type and a negative association with the other type of error. These results show that the two types of errors might indeed be different, which is in contrast to much of the previous literature that showed all medication errors as a single category under the implicit assumption that they were the same. While continued research should be focused on understanding the mechanism of error development and the etiology of each type of error, we suggest two possible explanations for these findings.

First, attributes of the source of medication error data must be acknowledged. For most hospitals in this study, voluntary incident reports, which are by nature subject to reporting bias, were the predominant source of data collection. When an error occurs, nurses can choose whether to report it, and their decision is often influenced by the obviousness of the error (Osborne, Blais, & Hayes, 1999). For example, severe medication errors, by the definition used in this study, are often too obvious to conceal and thus the number of reported cases is likely to be close to the actual number of severe errors. In contrast, nonsevere medication errors are often less obvious, and nurses might believe there is less necessity to report them. In other words, reported numbers of nonsevere errors are likely to be the result of nurses' error-reporting behavior, rather than actual incidences.

Another explanation might relate to a subjective classification criterion we used in categorizing each type of medication error. A severe medication error in one hospital or one nursing unit might not be classified as severe in another hospital or unit. For example, some nursing units in the sample had a hospital policy that required all medication errors, regardless of severity, to be treated with increased nursing observation and extra care. In such cases, all medication errors reported on the nursing unit were classified as "severe" per the definition of this study. Although the number of nursing units that reported such a policy was small (n=2), others might have had such a policy but did not report it.

One of the limitations of the study is that we did not have detailed patient-level clinical information that would have allowed us to control reliably for severity of illness. However, the sample was homogeneous, including medical units, surgical units, and joint medical-surgical units. In addition, we did control for patient age, prior health status, and hospitalization in the previous year, all of which are likely to be proxy measures for severity. Nevertheless, future research should incorporate a reliable and valid method to measure patient severity of illness.

Findings on nurses' expertise are consistent with those from previous studies (Dunphy & Williamson, 2004; Ericsson, Whyte, & Ward, 2007; Letnieux-Charles & McGuire, 2006). Expert nurses would be expected to make fewer errors than would less expert nurses because they are better at preventing a potential error by checking on a patient's laboratory data, detecting an adverse effect, and anticipating an adverse reaction related to the patient's pathophysiology (e.g., drug toxicity because of renal dysfunction; Eisenhauer, Hurley, & Dolan, 2007). In addition, considering the attributes of nonsevere medication errors discussed earlier, it is also possible that expert nurses are better able to distinguish medication errors with a low likelihood of harm. When they make an error with negligible harm to the patient, they believe the error is not going to have detrimental effects and, therefore, will be less likely to report them. In contrast, novice nurses might be more likely to report their errors regardless of the clinical significance of the error because they are not confident with their clinical skills and knowledge, and might not know what effect the error will have on a patient.

With regard to the findings on nurses' education levels, this is the first study to indicate empirical evidence that nurses' education has an effect on severe medication errors. It is also the first study we know of to show that there is an optimal proportion of BSN-prepared nurses to decrease medication errors, rather than assuming that "more is better." These findings are in contrast to those in Blegen, Vaughn, and Goode's study (2001) that showed no association between the percentage of BSN-prepared nurses and medication error rates.

An important difference between the two studies is that, while Blegen hypothesized a linear relationship, we specifically investigated whether a curvilinear relationship existed. Another difference is while Blegen and colleagues did not distinguish between severe and nonsevere medication errors, we separated them and found a significant association only for severe medication errors. Because most medication errors usually have inconsequential harm, if any, to patients, a significant portion of medication errors in studies about overall medication errors might be nonsevere. In other words, nurses' educational levels might have differential effects on severe and nonsevere medication errors.

We also found that nurses' experience was significantly and positively related to nonsevere medication errors. This finding contradicts a long-held belief that experienced nurses are less likely to make errors and also less likely to report them. For example, Blegen et al. (2001) found a significant, negative linear relationship between nurses' experience and medication errors. One possible explanation for this discrepancy is that as nurses gain experience, they might make more rule-based errors, which are the result of failure to apply a guiding principle (Reason, 1990). An example of rule-based medication errors is giving an intramuscular injection of diclofenac into the lateral thigh rather than the buttock, the preferred site for the drug, because the thigh is the usually preferred site for intramuscular injections in general (Ferner & Aronson, 2006).

Although no studies have been found in nursing, studies in other areas have indicated experienced workers' tendency to make rule-based errors. For example, in a field study of 198 clerical workers at 18 German organizations, Zapf, Brodbeck, Frese, Peters, and Prümper (1992) found that, while novices committed more knowledge- based errors, experienced workers made more rule-based errors or used correct actions in a wrong situation. Future research is warranted to better understand whether experienced nurses make more rule-based errors and, if so, how to develop management strategies for such cases.

We found that the greater the availability of medication-related support services, the more nonsevere medication errors were reported. Intuitively, one would expect that nursing units with more support systems should have fewer medication errors, regardless of error severity. For example, nursing units in which pharmacist consultations are readily available would be expected to have fewer medication errors than their counterparts as would those that have order transcribing service systems. However, as Reason (1995) discussed, instead of resolving human error problems, automation and increasing the numbers and types of advanced equipment might merely "relocate" problems. For example, nurses might make more errors when they have support services because, over time, they might become dependent on such services and, consequently, less careful when administering medications. Another possibility is that, assuming nonsevere medication errors are the reflection of error-reporting behaviors, nursing units where medication- related support services are available might be more likely to have a culture of reporting and, thus, have higher reported error rates.

Recommendations and Conclusions

Findings from this study provide the rationale for a new perspective on the different types of medication errors. Our results show that instead of all errors being the same, the two types of errors might differ, which will require different approaches to error prevention and management strategies. This study also warrants a thorough understanding of the role of medication-related support services on medication errors and the optimal levels of nurses' education and experience to decrease incidence of each type of errors. In fact, each type of antecedent examined in the current study might have different implications for different nursing units. Future research is needed to determine if the associations identified in the current study (performed in medical-surgical units) are true for other types of nursing units, e.g., ICUs, as patient characteristics and nursing care needed for patients differ by type of nursing unit.

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Clinical Resources

- National Coordinating Council for Medication Error Reporting and Prevention: http://www.nccmerp.org
- The Institute for Safe Medication Practices: http://www.ismp.org/
- Massachusetts Coalition for the Prevention of Medical Errors: http://www.macoalition.oig/

Table 1

Descriptive Statistics (*N* = 1,671)

	MEAN	***SD***	**Min**	**Max**
Dependent variables				
Severe medication errors	0.61	1.37	0	15.00
Nonsevere medication errors	3.86	4.71	0	38.00
Independent variables				
Work dynamics	26.84	3.50	15.79	37.40
RN hours	62.14	13.74	26.97	100.00
Communication with physicians	25.36	2.22	15.00	30.31
Nursing expertise	42.44	2.09	34.78	46.89
Education	36.52	19.36	0	100.00
Experience	138.49	45.38	43.57	322.80
Medication-related support services	8.83	1.19	3.71	11.35
Patient Factors				
Age	56.91	7.53	36.71	78.25
Health status	3.46	0.45	2.00	5.00
Previous hospitalization	0.53	0.21	0	1.00

Table 2

GEE Estimates and Z Statistics (N=1,671)

	Nonsevere errors		Severe errors	
Variable	**coefficient**	**Z**	**coefficient**	**Z**
Intercept	−4.0046 *	−2.43	−9.3970 ***	−3.65
Work-environment factors				
Work dynamics	−0.0122	−0.69	−0.0008	−0.03
RN hours	−0.0040	−0.87	0.0065	0.97
Team factors				
Communication with physicians	0.0349	1.45	0.0606	1.45
Nursing expertise	−0.0715 **	−2.59	0.0216	0.42
Person Factors				
Education	−0.0123	−1.49	−0.0432 ***	−3.34
Education^2	0.0001	1.29	0.0004 **	2.75
Experience	0.0112 **	2.71	0.0027	0.30
Experience squared	−0.0000 **	−2.74	−0.0000	−0.09
Medication-related support services	0.1221 **	2.87	−0.0592	−0.90
Patient factors				
Age	−0.0050	−0.76	0.0097	0.86
Health status	−0.0363	−0.26	−0.0577	−0.28
Previous hospitalization	0.2083	0.78	0.1613	0.41

Note.

* $p<0.05$;

** $p<0.01$;

*** $p<0.001$.